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## **Broad-range 16S rRNA gene polymerase chain reaction for diagnosis of culture-negative bacterial infections**

Rampini, S K ; Bloemberg, G V ; Keller, P M ; Büchler, A C ; Dollenmaier, G ; Speck, R F ; Böttger, E C

**Abstract:** **BACKGROUND:** Broad-range 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) is used for detection and identification of bacterial pathogens in clinical specimens from patients with a high suspicion for infection. However, prospective studies addressing the impact and clinical value of broad-range bacterial 16S rRNA gene amplification for diagnosis of acute infectious diseases in nonselected patient populations are lacking. **METHODS:** We first assessed the diagnostic performance of 16S rRNA gene PCR compared with routine bacterial culture. Second, we addressed prospectively the impact and clinical value of broad-range PCR for the diagnosis of acute infections using samples that tested negative by routine bacterial culture; the corresponding patients' data were evaluated by detailed medical record reviews. **RESULTS:** Results from 394 specimens showed a high concordance of >90% for 16S rRNA gene PCR and routine bacterial culture, indicating that the diagnostic performance of PCR for acute bacterial infections is comparable to that of bacterial culture, which is currently considered the gold standard. In this prospective study, 231 specimens with a negative result on routine bacterial culture were analyzed with PCR, and patients' clinical data were reviewed. We found that broad-range 16S rRNA gene PCR showed a sensitivity, specificity, positive predictive value, and negative predictive value of 42.9%, 100%, 100%, and 80.2% for culture-negative bacterial infections. **CONCLUSIONS:** This study defines the role of 16S rRNA gene PCR for diagnosis of culture-negative bacterial infections. Our data show that 16S rRNA gene PCR is particularly useful for identification of bacterial pathogens in patients pretreated with antibiotics.

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# Broad-Range 16S rRNA Gene Polymerase Chain Reaction for Diagnosis of Culture-Negative Bacterial Infections

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**Background.** Broad-range 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) is used for detection and identification of bacterial pathogens in clinical specimens from patients with a high suspicion for infection. However, prospective studies addressing the impact and clinical value of broad-range bacterial 16S rRNA gene amplification for diagnosis of acute infectious diseases in nonselected patient populations are lacking.

**Methods.** We first assessed the diagnostic performance of 16S rRNA gene PCR compared with routine bacterial culture. Second, we addressed prospectively the impact and clinical value of broad-range PCR for the diagnosis of acute infections using samples that tested negative by routine bacterial culture; the corresponding patients' data were evaluated by detailed medical record reviews.

**Results.** Results from 394 specimens showed a high concordance of >90% for 16S rRNA gene PCR and routine bacterial culture, indicating that the diagnostic performance of PCR for acute bacterial infections is comparable to that of bacterial culture, which is currently considered the gold standard. In this prospective study, 231 specimens with a negative result on routine bacterial culture were analyzed with PCR, and patients' clinical data were reviewed. We found that broad-range 16S rRNA gene PCR showed a sensitivity, specificity, positive predictive value, and negative predictive value of 42.9%, 100%, 100%, and 80.2% for culture-negative bacterial infections.

**Conclusions.** This study defines the role of 16S rRNA gene PCR for diagnosis of culture-negative bacterial infections. Our data show that 16S rRNA gene PCR is particularly useful for identification of bacterial pathogens in patients pretreated with antibiotics.

Bacterial infections remain a leading cause of death [1] and represent a massive financial burden to the health care system [2]. The traditional microbiological workup of clinical specimens is based on Gram staining, cultures on various media, and phenotypic identification schemes. Over the past 2 decades, molecular genetic techniques have been implemented for accurate pathogen

identification in diagnostic microbiology [2–10]. Broad-range 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) is used to detect and identify bacterial pathogens in clinical specimens [11–14] (Supplementary Table 1; online only), mostly in cases where bacterial infection is suspected but cultures remain negative [15–17]. Broad-range 16S rRNA gene PCR is particularly suitable for bacteria that are difficult to culture such as *Mycobacterium genavense* [18], *Tropheryma whippelii* [19], *Ehrlichia chaffeensis* [20], and *Coxiella burnetii* [21].

Despite the wide implementation of 16S rRNA gene PCR [22–27], there are few evidence-based studies addressing systematically its diagnostic impact in nonselected (random) patient populations, that is, in patients suspected to have infectious disease but not limited to particular disease entities. In addition, little information

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is available on how to effectively implement 16S rRNA gene PCR in a diagnostic workflow. Here, we performed both a prospective laboratory study to compare the diagnostic performance of PCR with bacterial culture and a prospective clinical study to assess the impact of broad-range PCR in the diagnosis of acute infection. In particular, we studied an algorithm that is based on a broad-range 16S rRNA gene PCR for culture-negative specimens from patients with suspected infectious disease.

## METHODS

This study was approved by the ethical committee of the canton of Zurich, Switzerland, and was done according to good clinical practice. Study design was composed of a laboratory study and a clinical study. In the laboratory study, specimens from primary sterile body sites were subjected (in parallel) to conventional microbiological culture (performed at the Institute for Clinical Microbiology and Immunology in St Gallen, Switzerland) and broad-range 16S rRNA gene PCR (performed at the Institute of Medical Microbiology in Zurich), to compare the diagnostic performance of PCR versus culture. In the clinical study, an algorithm was used integrating the broad-range PCR into the diagnostic sample workup. In this algorithm, samples from primary sterile body sites submitted to the microbiological laboratory were subjected to broad-range PCR if cultures (including enriched cultures) remained negative after 72 hours of incubation (all analyses were performed at the Institute of Medical Microbiology in Zurich). The clinical study is based on a prospective design as related to the microbiological workup of culture-negative specimens by 16S rRNA gene PCR; the patients' medical histories were reviewed retrospectively.

### Clinical Specimens

Patient samples were obtained from 2 tertiary care hospitals, the Kantonsspital St Gallen (KSSG) and the University Hospital of Zurich (USZ). KSSG is an 860-bed hospital in the eastern part of Switzerland; the samples from KSSG were used for the laboratory study. USZ is an 850-bed academic center; the samples from USZ were used for the clinical study.

### Medical Record Review

Clinical data of the patients enrolled in the clinical study were obtained by medical record review and analyzed for the likelihood of an infection. A panel of 2 senior infectious disease consultants (S. K. R. and R. F. S.) and 3 senior microbiologists (G. V. B., P. M. K., E. C. B.) discussed each case in depth and categorized it as definite, likely, unlikely, or no infection. Criteria included clinical signs and symptoms, chemistry parameters (eg, leukocyte count, C-reactive protein, procalcitonin), microbiological findings (eg, serology, previous culture results), radiological findings, previous antibiotic treatment,

and clinical diagnosis [28]. In the final analysis, we added patients with "likely infection" to the "definite infection" category, and we excluded patients with "unlikely infection" from the study to minimize false interpretations. In general, scoring of patients on the basis of medical record review was done blinded to the 16S rRNA gene PCR results. When data were not unequivocally clear, all data available, including the results of microbiological analysis, were considered. We defined all patients who received antibiotics, including peri-operative antibiotic prophylaxis, within 21 days prior to sampling as treated. This definition did not take into account whether the eventual microorganism identified was susceptible to the antibiotic prescribed.

## Microbiological Analyses

### Microscopy

Gram stains of clinical specimens were prepared according to standard procedures. The mean bacterial count was determined from ten 1000-fold visual fields (<5; 5–25; >25 bacteria per visual field) and the mean count for leukocytes from ten 100-fold magnified fields (1–9; 10–25; >25 leukocytes per field).

### Cultures

Cultures were performed as described elsewhere [7, 8]. In brief, solid and tissue samples were placed in sterile 0.9% sodium chloride solution following retrieval, minced upon receipt in the laboratory using an Ultra-Turrax tissue homogenizer (IKA), inoculated for culture, and incubated under aerobic and anaerobic conditions. An anaerobic glove box (DW Scientific) with prereduced anaerobic media was used for anaerobic culturing conditions. Aerobic media included Columbia blood agar, MacConkey agar, colistin nalidixic acid (CNA) blood agar, and Crowe agar [29]. Anaerobic media included *Brucella* agar, kanamycin-vancomycin agar, and phenylethyl alcohol agar. Thioglycolate broth was used for enrichment cultures. Agar plates were examined for growth after 24, 48, and 72 hours. Optical turbidity of the liquid enrichment medium was inspected after 1, 2, 3, and 10 days of incubation. Cultures were considered negative if no bacterial growth was visible after 10 days of incubation. For the laboratory study, this period was extended to 14 days. Liquid samples were inoculated into aerobic and anaerobic BacT/Alert Blood Culture flasks (BioMérieux) for enrichment cultures and incubated in a BacT/Alert system for 6 days. Flasks with a positive growth signal were subcultured using the set of agar-based media described above.

### DNA Extraction and Polymerase Chain Reaction

DNA extraction and 16S rRNA gene PCR was done as described previously [7]. *Escherichia coli* chromosomal DNA was used as a positive control. As negative control buffers, PCR reagents and elution column solutions were routinely tested for bacterial DNA contamination. If bacterial identification in a clinical sample was identical to the bacterial DNA contamination in 1 of

the negative controls (<5% of the samples), this sample was considered PCR negative. 16S rRNA gene homology analyses were done using the SmartGene IDNS database and software [30] (SmartGene).

### Statistical Methods

The statistical methods used are described in the text or in the figure legend related to the dataset analyzed. Statistical calculations were done using GraphPad Prism software, version 5.02 (GraphPad Software).

## RESULTS

### Sensitivity and Specificity of Broad-Range 16S rRNA Gene Polymerase Chain Reaction Compared With Conventional Culture (Laboratory Study)

To determine the minimal number of bacterial chromosomal copies, serial dilutions of *E. coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) DNA were subjected to broad-range 16S rRNA gene PCR. The detection limit of the procedure using polyacrylamide gel electrophoresis and silver staining to resolve 16S rRNA gene PCR amplicons was consistently 40 gene copies per reaction.

We prospectively assessed 16S rRNA gene PCR compared with conventional culture. The study period was from July 2008 to December 2008 and included 394 samples from primary sterile body sites of a nonselected patient population. In this laboratory study, clinical data were not taken into account. There was a high concordance of both culture and PCR in 357 of 394 (90.6%) analyses (Tables 1 and 2). Discordant results were observed in 37 cases with 18 (4.6%) culture-negative, PCR-positive results and 19 (4.8%) culture-positive, PCR-negative results. Species assignments in the 18 culture-negative, PCR-positive samples (Table 2) included *Staphylococcus* spp (n = 5), *Streptococcus* spp (n = 3), *Ralstonia* spp (n = 2), *Acinetobacter* spp (n = 2), *Burkholderia* sp (n = 1), *E. coli* (n = 1), *Citrobacter freundii* (n = 1), *Haemophilus influenzae* (n = 1), *Porphyromonas asaccharolyticus* (n = 1), and *Peptinophilus* sp (n = 1). *Ralstonia* spp (n = 2), *Acinetobacter* spp (n = 2), and *Burkholderia* spp (n = 1) were considered to represent laboratory or sample retrieval

contaminants (5 of 104 PCR-positive results). The isolates identified in the 19 culture-positive, PCR-negative specimens (Table 2) included *Propionibacterium acnes* (n = 11), coagulase-negative staphylococci (n = 5), *S. aureus* (n = 2), and *Staphylococcus lugdunensis* (n = 1). Fifteen of 19 culture-positive, PCR-negative samples had only very low numbers of bacteria present as indicated by the observation that these samples showed bacterial growth only after several days of incubation in enriched liquid culture medium (Table 2).

### Broad-Range 16S rRNA Gene Polymerase Chain Reaction for Diagnosis of Infections in Culture-Negative Samples (Clinical Study)

In the clinical study, we prospectively enrolled all patient specimens received from July 2008 to December 2008 (n = 231; Figure 1) for which bacterial cultures had remained negative for growth after 3 days of incubation. The likelihood of infection was retrospectively judged on the basis of clinical and laboratory data. Data were obtained by detailed patient medical record review. Incorporating the different parameters resulted in a final categorization of definite infection or no infection.

Thirty-two samples were excluded because of unavailable or insufficient patient documentation or because cultures became positive after prolonged incubation (Figure 1). Five of 29 culture-negative, PCR-positive and 8 of 170 culture-negative, PCR-negative samples were excluded because no firm clinical assignment was possible (Figure 1). In total, 186 specimens were included in the analysis: aspirates and biopsies (n = 97), cerebrospinal fluids (n = 40), tissues (n = 21), heart valves (n = 11), wound swabs (n = 11), abscess materials (n = 4), and ascites (n = 2).

Twenty-four of 186 samples were PCR positive and included the following species: *Streptococcus* spp (n = 6), *S. aureus* (n = 4), *Ureaplasma urealyticum* (n = 3), *Staphylococcus epidermidis* (n = 3), *C. burnetii* (n = 2), *Porphyromonas endodontalis* (n = 3), *Fusobacterium nucleatum* (n = 1), species of the family Enterobacteriaceae (n = 1), *Enterococcus faecalis* (n = 1), and *Parvimonas micra* (n = 1) (Table 3). All of the 24 PCR-positive samples were from patients categorized as having definite infection. Of 186 samples, 162 were negative by broad-range PCR, including 32 patients with definite infection and 130 patients considered to have no infection based on detailed medical record reviews (Figure 1). The sensitivity of broad-range 16S rRNA gene PCR in correctly diagnosing bacterial infection for culture-negative samples was 42.9 %, the specificity was 100%, the positive predictive value (PPV) was 100%, and the negative predictive value (NPV) was 80.2% (Supplementary Table 2.1; online only).

Seventy-nine of 186 specimens were obtained from patients who had previously been treated with antibiotics or who were undergoing antibiotic treatment when the clinical specimen was

**Table 1. Broad-Range 16S rRNA Gene PCR Compared With Conventional Culture**

		Culture	
		+	–
PCR	+	86 (21.8%)	18 (4.6%)
	–	19 (4.8%)	271 (68.8%)

A total of 394 clinical specimens were included in the study. For bacterial identification see Table 2.

Abbreviations: PCR, polymerase chain reaction; –, negative; +, positive.

**Table 2. Compilation of Pathogens Identified by Broad-Range 16S rRNA Gene Sequencing and/or Culture in the Laboratory Study**

Species in culture-positive, PCR-positive specimens	No.	Species in culture-negative, PCR-positive specimens <sup>a</sup>	No.	Species in culture-positive, PCR-negative specimens <sup>b</sup>	No.
<i>Cupriavidus metallidurans</i>	1	<i>Acinetobacter johnsonii</i>	1	<i>Propionibacterium acnes</i>	11 <sup>c</sup>
<i>Enterobacter cloacae</i>	1	<i>Acinetobacter</i> sp	1	<i>Staphylococcus lugdunensis</i>	1
<i>Enterobacter</i> sp	10	<i>Burkholderia</i> sp	1	<i>Staphylococcus aureus</i>	2
Enterobacteriaceae	3	<i>Citrobacter freundii</i>	1	<i>Staphylococcus capitis</i>	1
<i>Enterococcus faecalis</i>	1	Enterobacteriaceae	1	<i>Staphylococcus epidermidis</i>	3
<i>Enterococcus faecium</i>	1	<i>Haemophilus influenzae</i>	1	<i>Staphylococcus</i> sp	1
<i>Morganella morganii</i>	2	<i>Peptoniphilus</i> sp	2		
Paenibacillaceae	1	<i>Porphyromonas asaccharolyticus</i>	1		
<i>Proteus</i> sp	1	<i>Ralstonia insidiosa</i>	1		
<i>Proteus vulgaris</i>	3	<i>Ralstonia pickettii</i>	1		
<i>S. aureus</i>	32	<i>S. aureus</i>	2		
<i>S. epidermidis</i>	19	<i>S. epidermidis</i>	1		
<i>Streptococcus agalactiae</i>	5	<i>Staphylococcus</i> sp	2		
<i>Streptococcus dysgalactiae</i>	1	<i>S. agalactiae</i>	1		
<i>Streptococcus</i> sp	5	<i>Streptococcus infantis</i>	1		
		<i>Streptococcus</i> sp	1		
<b>Total</b>	<b>86</b>	<b>Total</b>	<b>18</b>	<b>Total</b>	<b>19</b>

Abbreviation: PCR, polymerase chain reaction.

<sup>a</sup> *Ralstonia* spp, *Acinetobacter* spp, and *Burkholderia* spp were considered laboratory or sample retrieval contaminations (5 of 104 PCR-positive results).

<sup>b</sup> Fifteen of 19 specimens were positive by culture only following prolonged incubation (>48 hours) with monitoring up to 14 days in enriched broth medium; 1 of 19 specimens was positive by culture with a single colony-forming unit; 3 of 19 specimens were positive by culture within 48 hours of incubation (for these 3 samples the corresponding isolate was identified by PCR in a second specimen of the respective patient).

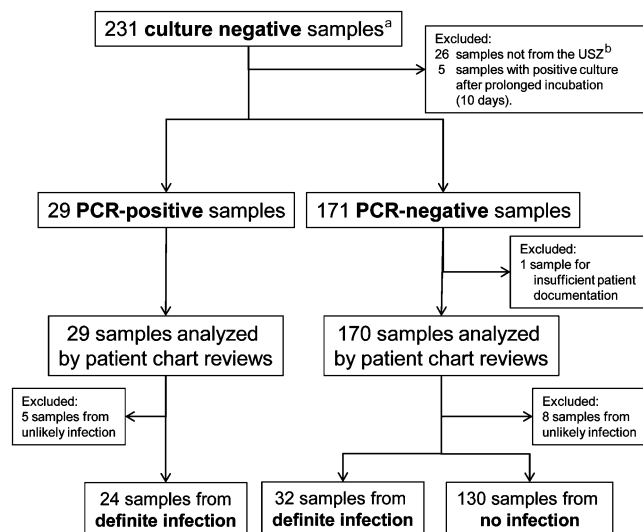
<sup>c</sup> Four cultures became positive after >10 days of incubation.

obtained. It is noteworthy that all 24 specimens with culture-negative, PCR-positive results (Table 3) were obtained from this patient group. Thirty-one of 55 specimens of the antibiotic-treated patients with culture-negative, PCR-negative results were categorized as definite infection. The sensitivity of broad-range 16S rRNA gene PCR in correctly diagnosing bacterial infection

for culture-negative samples from patients undergoing or having previously undergone antibiotic therapy was 43.6 %, the specificity was 100%, the PPV was 100%, and the NPV was 43.6 % (Supplementary Table 2.2; online only).

There was a single patient with a clinically well-defined infection who was antibiotic naive and whose sample culture was 16S rRNA gene PCR negative (Supplementary Table 2.3; online only). This case was eventually identified as *Mycobacterium chelonae* infection by *Mycobacterium* genus-specific PCR. All other specimens from antibiotic-naive patients (106 samples) were from cases we categorized as no infection.

Five of 24 culture-negative, PCR-positive specimens (Table 3) were obtained from patients recovered from infectious endocarditis (IE) following antibiotic therapy (samples 1, 2, 7, 12, and 21; Table 3). There was complete agreement between the culture results from the specimens obtained before antibiotic treatment and PCR results obtained when the heart valve was replaced during antibiotic treatment. Two of 24 specimens with culture-negative, PCR-positive results were from 2 patients who had perioperative prophylaxis at the time the specimen was retrieved (samples 3 and 18; Table 3). The remaining 17 culture-negative, PCR-positive samples were retrieved from patients who had been treated with antibiotics for various periods of time. To assess a possible impact of the duration of antibiotic therapy on pathogen detection by PCR, a comparison was made for PCR-positive (n = 24) and PCR-negative (n = 31) specimens from



**Figure 1.** Enrollment of specimens in the clinical study. <sup>a</sup>Culture negative for 3 days. <sup>b</sup>USZ, University Hospital Zürich. PCR, polymerase chain reaction.



**Table 3. Summary of Culture-Negative, Polymerase Chain Reaction-Positive Specimens in the Clinical Study**

Clinical specimen	Bacteria observed by microscopy (+, ++, +++ <sup>a</sup> )	Leukocytes observed by microscopy (+, ++, +++ <sup>b</sup> )	Bacterial species identified by 16S rRNA gene PCR	Days after end of antibiotic therapy/days undergoing antibiotic therapy <sup>c</sup>
1 Aortic valve	nd	+	<i>Streptococcus mutans</i>	0/32
2 Mitral valve	nd	++	<i>Enterococcus faecalis</i>	0/54
3 Deep wound (swab)	++ (Gram-positive cocci)	+++	<i>Parvimonas micra</i>	0/1
4 Cerebrospinal fluid	++ (Gram-positive cocci)	+++	<i>Staphylococcus aureus</i>	0/2
5 Sternal wound (swab)	nd	+++	<i>Staphylococcus epidermidis</i>	0/3
6 Cerebrospinal fluid	+++	nd	<i>S. epidermidis</i>	0/16
7 Mitral valve	nd	+	<i>S. aureus</i>	0/48
8 Abscess (brain) <sup>d</sup>	nd	nd	<i>Fusobacterium nucleatum</i> <i>Porphyromonas endodontalis</i>	0/7
9 Abscess (brain) <sup>d</sup>	nd	nd	<i>P. endodontalis</i>	0/7
10 Aspirate (shoulder)	nd	+++	<i>Streptococcus dysgalactiae</i>	20/39
11 Abscess (brain)	++ (Gram-positive cocci)	+++	<i>Streptococcus intermedius</i>	0/12
12 Aortic valve	+++ (Gram-positive cocci)	+	<i>Streptococcus</i> sp ( <i>Streptococcus mitis</i> group)	0/2
13 Pleural effusion	nd	+++	<i>P. endodontalis</i>	0/28
14 Sternal wound (swab)	nd	+	<i>S. epidermidis</i>	0/49
15 Aspirate (knee)	nd	+++	Enterobacteriaceae	0/5
16 Tissue	nd	+++	<i>S. aureus</i>	0/3
17 Tissue	nd	+++	<i>S. aureus</i>	0/3
18 Abscess (psoas muscle)	nd	++	<i>Coxiella burnetii</i>	0/1
19 Tissue (aneurysm)	nd	+	<i>C. burnetii</i>	0/7
20 Aortic valve	nd	+++	<i>Streptococcus</i> sp. ( <i>S. mitis</i> group)	21/42
21 Mitral valve	nd	+	<i>S. mitis</i>	0/25
22 Sternal wound (swab)	nd	+	<i>Ureaplasma urealyticum</i>	0/4
23 Sternal wound (swab)	nd	+	<i>U. urealyticum</i>	0/4
24 Sternal wound (swab)	nd	++	<i>U. urealyticum</i>	0/4

Abbreviations: nd, not detected; PCR, polymerase chain reaction; pos, positive; rRNA, ribosomal RNA.

<sup>a</sup> Bacterial cell count in ten 1000-fold visual fields: +, <5; ++, 5–25; +++, >25.

<sup>b</sup> Leukocyte count in ten 100-fold magnified fields: +, 1–9; ++, 10–25; +++, >25.

<sup>c</sup> 0/32 indicates that the specimen was obtained while the patient was under antibiotic therapy and had received antibiotic therapy for the past 32 days; 20/39 indicates that the specimen was retrieved 20 days after an antibiotic therapy of 39 days; 0/1 indicates that the specimen was retrieved at the start of antibiotic therapy.

<sup>d</sup> This case was recently published in detail [17].

patients with acute infections treated with antibiotics for various periods of time. For simplicity, we categorized clinical specimens into groups obtained within the first 10 ( $n = 28$ ), 20 ( $n = 11$ ), 30 ( $n = 5$ ), 40 ( $n = 5$ ), 50 ( $n = 4$ ), and 60 ( $n = 2$ ) days after start of antibiotic treatment. Similar numbers of positive and negative PCR results were observed in all specimen groups, indicating that PCR positivity is independent of the duration of antibiotic treatment (at least up to 60 days).

## DISCUSSION

Clinicians are frequently challenged with the suspicion of acute infectious diseases even when a conventional microbiological workup remains negative. Broad-range PCR detects many clinical relevant bacteria as documented by the bacterial assignments

obtained during 2005–2008 (Supplementary Table 1; online only). The primary goal of this study was to assess the value of 16S rRNA gene PCR for the diagnosis of acute bacterial infections in a nonselected patient population, in particular, to provide evidence-based data to the clinician for a targeted use of broad-range 16S rRNA gene PCR.

In the laboratory part of the study, we analyzed the diagnostic sensitivity and specificity of broad-range PCR in 394 specimens compared with routine bacterial culture. We found that the concordance between 16S rRNA gene PCR and culture is >90%. The sensitivity of broad-range PCR in our dataset was 81.9% and the specificity 93.8%. A recent study reported a sensitivity of 93% and a specificity of 72% for 16S rRNA gene PCR compared with conventional culture [31]. The differences in sensitivity and specificity between our

study and the earlier study most likely reflect the parallel workup of samples retrieved from primary sterile compartments by both culture and broad-range PCR in our study, that is, the nonselective use of 16S rRNA gene PCR. The high concordance between culture and PCR indicates a similar analytical sensitivity.

In the clinical part of this study, we assessed the diagnostic power of broad-range 16S rRNA PCR in the diagnosis of acute bacterial infections for which conventional cultures were negative and the likelihood of infection was assessed. Notably, the majority of samples submitted for microbiological analysis (130 of 186) were obtained from patients without firm evidence of infection as judged by retrospective medical record review. This high number of patients without infection reflects the difficulty of accurately diagnosing infection at the bedside, emphasizing the need of an interdisciplinary approach toward accurate diagnosis in which the clinical presentation, the laboratory findings, and the imaging all contribute to reach the final diagnosis.

The importance of 16S rRNA gene PCR is demonstrated in the clinical part of the study, where a pathogen was identified in 42.9% of the culture-negative specimens of patients with clear evidence of an infection as indicated by detailed medical record review (Table 3). The relatively high number of specimens with culture-negative, PCR-negative results reflects the prevalence of patients with sterile inflammatory processes (eg, elevated leukocyte counts in joint aspirates due to rheumatoid arthritis). The PPV of the 16S rRNA gene PCR was 100%, indicating that a positive result accurately identifies an infection. We are aware that a specificity and a PPV of 100% of broad-range PCR in the clinical study should be viewed with caution, as 5 of 104 positive PCR results in the laboratory study were thought to be due to environmental contaminants. Due to restrictions inherent in a clinical study design (ie, the necessity to exclude patients for whom no firm diagnosis could be established), we had to eliminate 5 of 29 PCR-positive and 8 of 170 PCR-negative samples from the clinical study (Figure 1). If we include these samples in the no-infection category, the specificity, PPV, and NPV of broad-range PCR are 96.5%, 82.8%, and 81.2%, respectively. A subanalysis revealed that the NPV was 99.1% for the patients who had not received antibiotics prior to sampling.

In the clinical study, 16S rRNA gene PCR was positive exclusively in patients who had previously undergone antibiotic treatment. This finding, together with the similar analytical sensitivity of culture and PCR as found in the laboratory study, suggests that carrying out 16S rRNA gene PCR and bacterial culture in parallel in antibiotic-naïve patients will not aid substantially in diagnosing infections. The vast majority of antibiotic prescriptions (>90%) are in the outpatients [32], and a substantial number of patients suffering from bacterial infections will have received antibiotics prior to hospital admission. This patient group frequently poses a diagnostic dilemma, as the cultures of

the causative infectious pathogen often remain negative. For this patient group, broad-range 16S rRNA PCR is particularly valuable and may add key diagnostic findings to improve patient management.

We also analyzed a possible influence of the length of antibiotic prescription on the detection of pathogens by 16S rRNA gene PCR, as we assumed that PCR positivity will decline with the duration of antibiotic therapy preceding clinical specimen retrieval. However, the ratio of PCR-positive and PCR-negative samples was independent of the length of prior antibiotic therapy (as analyzed for a maximum period of 60 days).

The majority of pathogens identified in the culture-negative, PCR-positive group of the clinical study were common pathogens, which usually grow without any particular requirements. There were 2 exceptions in this part of the study (Table 3): *C. burnetii* (recovered twice), which exclusively grows in cell culture or needs to be inoculated into laboratory animals [21], and *U. urealyticum* (recovered 3 times), which requires cultivation on selective medium for Mycoplasmataceae (retrospective inoculation of selective medium demonstrated the presence of *U. urealyticum* in all 3 samples). It goes without saying that common pathogens were more frequently found in the clinical study, as the number of infections with difficult-to-culture pathogens is low compared with common pathogens (see also Table 1; online only). From a clinical point of view, however, it is as important to recognize infections with unusual pathogens in a reliable manner as those due to common pathogens.

In summary, this study shows that there is a high concordance between broad-range 16S rRNA gene PCR and culture in pathogen detection and that broad-range PCR is particularly valuable for patients under antibiotic therapy. On the basis of our data, we propose that broad-range PCR of samples from primary sterile body sites should be done for patients with a high clinical suspicion of infection and negative culture results. The suspicion for infections with difficult-to-culture pathogens justifies a broad-range PCR on its own, in particular when a species-specific molecular test is not available. Implementing an algorithm that excludes culture-positive samples is both diagnostically and economically feasible.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online ([http://www.oxfordjournals.org/our\\_journals/cid/](http://www.oxfordjournals.org/our_journals/cid/)). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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